4500
FS-SO-2203-1.32
(Problem 2)

November 1974
Study Plan Summary
T. J. Perry - Pineville, La.

# INDUCTION OF FRUITING IN AN UNIDENTIFIED BASIDIOMYCETE, A MYCANGIAL SYMBIONT OF THE SOUTHERN PINE BEETLE

A basidiomycete has been consistently isolated from the mycangium of the southern pine beetle. Its symbiotic relationship is thought to be nutritionally beneficial, supplying one or more nutrients to the beetle during its development.

The identity of this fungus has been an enigma. When squeezed from the mycangia, the growth resembles previously described ambrosial fungus species found associated with bark and ambrosia beetles, but still is inconsistent with described data. On artificial media, the fungus produces clamp connections typical of basidiomycetes. The identification is not only hindered by the two distinctly different growth forms, but also because it has never fruited in culture.

This study will attempt to induce fruiting through mechanical injury on specialized media, a technique successfully used with other basidiomycetes, in the hope of generating enough morphological and physiological data to aid in the identity of this fungus.

**UNITED STATES DEPARTMENT OF AGRICULTURE**
**FOREST SERVICE**

SO

*REPLY TO:* 1630 Written Information

November 11, 1974

*SUBJECT:* Study Plan, FS-SO-2203-1.32

*TO:* T. Harrington, AD

Attached is a copy of Study Plan FS-SO-2203-1.32 and summary by Thelma J. Perry. I request a waiver of biometrics review.

Stan Barras

STANLEY J. BARRAS
Acting Project Leader
RWU-2203

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STUDY PLAN

# INDUCTION OF FRUITING IN AN UNIDENTIFIED BASIDIOMYCETE, A MYCANGIAL SYMBIONT OF THE SOUTHERN PINE BEETLE

Prepared by:

Thelma J. Perry
Biological Laboratory Technician
(Microbiology)

11/8/74
(Date)

Approved:

STANLEY J. BARRAS
Acting Project Leader
RWU-2203

11/8/74
(Date)

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# STUDY PLAN

# INDUCTION OF FRUITING IN AN UNIDENTIFIED BASIDIOMYCETE, A MYCANGIAL SYMBIONT OF THE SOUTHERN PINE BEETLE

## INTRODUCTION

Two fungi have consistently been isolated from the mycangium of the southern pine beetle (Barras and Perry 1972). One has been identified as a Ceratocystis imperfect stage, Sporothrix schenckii. The other, a basidiomycete, has presented problems in its identification. These fungi are important to the successful development of the insect.

The basidiomycete exists in two forms. When squeezed from the mycangium, the ambrosial form is apparent. It proliferates in the mycangium as globular, chlamydosporous, short chained hyphae. When inoculated onto media, the fungus has the characteristics of a slow-growing filamentous fungus. It produces a brown exudate, clamp connections and sterile mycelia. It has never fruited in culture.

The purpose of this study is to attempt to induce fruiting by mechanical injury on chemically defined media and thus identify the fungus (Leonard and Dick 1973).

## METHODS AND MATERIALS

The fungus (known as SJB 122) will be isolated from the mycangia of southern pine beetles and inoculated on a basic medium containing 20.0 g dextose, 2.0 g peptone, 0.5 g $MgSO4-7H_2O$, 1.0 g. $K_2HPO_4$, 120 ug thiamine HCl and 20.0 g agar per liter of distilled water.

Four plates of the above medium will be inoculated to serve as transfer inoculum. They will be incubated at 25°C and allowed to grow for 2 weeks and/or until they have attained a diameter of at least 5 cm.

Three modifications of the basic medium will also be utilized consisting of (1) only 1 g peptone, (2) the addition of 1.0 g yeast extract and (3) dl-asparagine substituted for peptone. All media are sterilized by autoclaving 20 minutes at 121°C. Dextrose will be sterilized separately to minimize carmelization.

The experiment will consist of 4 replicates of each of the four media (BM, 1/2 PBM, BMYE, BMA). The inoculum, a 10 mm plug, will be obtained from the basic medium previously inoculated and grown two weeks. The 10 mm plug will be placed inside a previously removed 15 mm plug of the test media. This will give an air space of 5 mm and allow a minimum amount of nutrition to be obtained from the original media.

The plates will be incubated for two weeks at 25°C to allow the relatively slow-growing mycangial fungus to attain a diameter of at least 4 cm.

Colonies of the fungus on the above media will be mechanically injured by localized cutting with a sterile scapel. The cutting will be uniform, a 90 degree angle from center to edge, on all plates. The plates will be reincubated and observed every three days for the production of fruiting bodies. If the fungus responds to these media by producing fruit bodies, available basidiomycete keys will be used for identification.

## LITERATURE CITED

Barras, S. J. and T. Perry.

1972. Fungal Symbionts in the prothoracic mycangium of Dendroctonus frontalis (Coleopt.:Scolytidae). Z. Ang. Entomol. 71:95-104.

Leonard, T. J. and S. Dick.

1973. Induction of haploid fruiting by mechanical injury in Schizophyllum commune. Mycologia. 65:809-822.

## ESTIMATED TIME

6-8 weeks

## PERSONNEL

Thelma J. Perry, Microbiological Laboratory Technician